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THE VITAMIN B COMPLEX*

BY

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It should be emphasized that research with controlled experiments upon animals is our only chance of making real and basic progress in nutritional problems. Such research in many laboratories has shown that at least six entities compose the vitamin B complex, which can be described as follows:

Vitamin B_1 (aneurin or torulin[1])
Vitamin B_2[2] < lactoflavin[6] / vitamin B_6[7] (rat anti-dermatitis)
Vitamin B_3[3] and B_5[5] (including the chicken anti-pellagra factor[8])
Vitamin B_4[4] (position at present obscure)
Human anti-pellagra, P.P.
Dog black tongue factor
Pigeon heart-block factor
Bios and growth factors for micro-organisms

Of these, vitamin B_1 (also known as aneurin and torulin), lactoflavin, and vitamin B_6 have been defined more completely than others, the last two being components of the original vitamin B_2. Several of the other factors are clearly of interest; to those whose activities embrace the poultry yard the bird factors will be of economic importance. With the exception of vitamin B_1 the position of the other factors in human nutrition is not yet known, but it is extremely unlikely that some are not essential; there is a widespread feeling that the problem of human pellagra is bound up with some of the other parts of vitamin B. Vitamin B_1[9] and lactoflavin are the only ones which have been obtained pure, and lactoflavin[10] is the only one that has been synthesized, though the synthesis of vitamin B_1 is reported to be a not far distant event.

The failure to advance more quickly with work in this group of vitamins has not been due to lack of effort by biochemists and other investigators, but to the extreme difficulty of the experimental work. I should like to make the point that, even under carefully controlled laboratory conditions, it has often proved impossible to repeat observations of an apparently simple nature. There are two practical lessons for nutritional medicine to be learnt from these laboratory experiences; one of these elaborates some remarks by the opener upon the importance of soil in the growth of foodstuffs.

1. Foodstuffs which appear to be the same may be actually different owing to soil and temperature conditions of growth, or to the differing diets of farm animals. The vitamin B content of milk, as an illustration, may differ considerably. The nine foodstuffs must be of a proper standard composition, and it is not safe to consider anyone properly fed unless the food actually eaten is first-rate. Much research is in progress on these lines. The food eaten may appear to contain enough vitamin B_1, but quantitative analysis may reveal that the amounts prove too little. Cowgill[11] quotes a good illustration of this. We must learn to think quantitatively as well as qualitatively.

2. The second point which stands out from some animal experiments with vitamin B_1 is that there is idiosyncrasy in the requirement of some factors. Such a view has direct clinical support for vitamin D. It is of wide importance because it may easily mean that different races require rather different nutritional treatment as well as individuals. I would alter the popular saying to run: "One man's meat is not necessarily another's full meal."

The only important practical point to make here is that there is, of course, a basic minimum for all, but that it is unsafe to consider that a diet is adequate for a given individual merely because a large number of other individuals happen to thrive upon it. The clinician should always ask himself the question whether the case before him happens to be one of dietary idiosyncrasy to some factor; I believe this to be much more common than is usually supposed. The exact knowledge of the intimate action of the vitamins lies in the tissues; the biochemical reactions which they catalyse will doubtless help us ultimately to understand the reasons for these results, and to apply our knowledge more intelligently. A few words upon the nature and action of some of these B factors may now be given.

Nature of the B Factors

Vitamin B_1 is a pyrimidine-thiazole (sulphur-nitrogen-carbon ring) compound, according to Williams and colleagues. It has been isolated as a white crystalline solid, usually in the form of the hydrochloride. It can be tested with animals, and its deficiency causes convulsions of a particular type. It gives improvement in appetite, assists growth in young animals, and helps to maintain health in adults; in vitamin B_1-deficient rats it is a cure for a specific bradycardia.[12] Approximately 0.01 mg. is all that is necessary for cure of a pigeon. The compound gives a specific azo reaction.[28]

Two theories of its function have been proposed in the past: (*a*) that it is concerned with tissue oxidations, and (*b*) that it is concerned with carbohydrate metabol-

* A communication to the Section of Nutrition at the Annual Meeting of the British Medical Association, Oxford, 1936. Since it was written Vitamin B_1 has been synthesized.

ism.[13] Recent research has proved that both these views are correct. At first the hypothesis that oxidation of the intermediary carbohydrate metabolite lactic acid, ($CH_3CHOH.COOH$) was affected by vitamin B_1 received some support ; lately this has been discarded in favour of the pyruvic acid theory. Pyruvic acid ($CH_3.CO.COOH$) is a near relative of lactic acid, and is now believed to be an essential intermediate in carbohydrate degradation.

According to the pyruvic acid theory of vitamin B_1, which we have elaborated in the Biochemical Laboratory at Oxford, this vitamin is an essential catalyst in its oxidation. When this vitamin catalyst is missing from tissues the energy metabolism fails, and so the cell does not function. Failure in brain cells leads to convulsions. I need not mention much of the evidence, which is extensive—merely two points: vitamin B_1 can be shown *in vitro* to have a specific catalytic effect upon the brain tissue of pigeons suffering from this deficiency. Pyruvic acid accumulates in the blood of vitamin B_1-deficient animals (and probably men).

This theory of B_1 action explains much that has been found in experimental " beri-beri " animals, and there are sufficient points of contact with human beri-beri to give confidence in its application to man. The lactic acid accumulations in blood (and tissues), which are a marked feature of experimental B_1 deficiency and human beri-beri, are explained by the suggestion that the oxidation of lactic acid is inhibited at the pyruvic acid stage. Lactic acid $\xrightarrow{C}$ pyruvic acid $\xrightarrow{D}$ further oxidation products, stage D occurring only in the presence of vitamin B_1.

I think personally that it is this interference with the cells of the lower brain which induces the gross changes in carbohydrate metabolism (alterations in carbohydrate tolerance and the like) as well as the well-known failures of temperature regulation which take place in the terminal stages (the oedemas are probably secondary). Not yet explained is the failure of appetite, which is the earliest and a very constant feature of a vitamin B_1-deficient diet, and has been much investigated by Cowgill and colleagues. The adult has been calculated to require some 250 to 500 international units (cf. [14]) of vitamin B_1 (about 1 mg. crystalline vitamin) ; the richest sources of vitamin B_1 are vitamin-enriched yeast, wheat-germ, pork, liver, kidney, and legumes.[15]

It is best, of course, to ensure that adults and children take enough vitamin B_1 for health. In every case of appetite failure, in my opinion, the vitamin B_1 content of the diet should be considered. This is one of the cases in which concentrated vitamin B_1 must be given ; ordinary sources are often too dilute for such a purpose. It is hardly necessary to say that commercial vitamin propaganda should not be taken at its face value, but that care should be taken to see that the strength of vitamin products issued has been properly assured by the British Pharmaceutical Society. Yeast varies enormously in vitamin B_1 potency. It is relevant here to note that both weight and appetite in children have been stated to be improved by giving a satisfactory yeast preparation (Summerfelt and Brown).[16]

Deficiency of Vitamin B_1

It is clear that gross deprivation of the vitamin B complex on a large scale may exist in other countries, but not in this one. Have we any evidence that mild vitamin B_1 deficiency may exist here? There are methods for obtaining concrete information to support what used to be mere opinion. The evidence comes first from urine and secondly from blood. If the theory is right that the brain and the body cells require a constant supply of vitamin B_1 in the blood, then B_1 deficiency should be associated with a fall of vitamin B_1 in the blood ; further, a change should show in the urine—less should be excreted. Taking the latter point first, the excretion of vitamin B_1 in urine has been studied recently by Helmer,[17] Harris *et al.*,[14] and Roscoe.[18] The results are a little contradictory, but agree in showing less excretion when there is vitamin B_1 deficiency.

More significant but more difficult is the study of the concentration in the blood, which has only just started. Meiklejohn has applied Schopfer's test to blood. The point of the test is that conditions can be so arranged that the growth of a small mould, *Phycomyces blakesleeanus*, is specifically catalysed by minute amounts (less than 0.001 mg.) of vitamin B_1. Meiklejohn's recent unpublished results prove that with vitamin B_1 deficiency there is a marked decrease in vitamin B_1 in the blood of pigeons, from 27γ to 5.5γ per 100 c.cm. (10γ B_1 = 5 international units). He is now applying this to patients, and it so happens that one case (from Dr. Ungley) of pyloric stenosis with polyneuritis gives a figure very much below any other case. Much more must be done to establish human normals, for which the present values are 8.5γ ± 1.0 per 100 c.cm. of blood (4 units)—that is, only one-third the amount in the pigeon ; with urine and blood tests available, accurate information should soon be reached.

Before concluding this section may I suggest the necessity of final controls by the injection of vitamin B_1. Other methods are likely to fail, especially if part of the trouble be due to interference with absorption in a deranged intestine.

In 1933[19] I wrote: " We should look for the use of vitamin B_1 in conditions of loss of appetite, oedema, palpitation and breathlessness, especially where there is defective removal of blood lactic acid after exercise, neuritic conditions, and painful muscles." This statement has now received support clinically. I can quote here *inter alia* that Vorhaus, Williams, and Waterman[20] have treated some 100 cases of various forms of neuritis with crystalline vitamin B_1 in doses up to 10 mg. ; only 8 per cent. failed to react. Tenderness disappeared as the pain improved ; there was relief of the weakness, while loss of appetite and paraesthesia were markedly benefited. In Oxford, Drs. Hobson, Cooke, and others have observed improvements in oedema, and there is other clinical evidence now in relation to alcoholic polyneuritis. (Ritchie Russell.[21])

Tests for Vitamin B_1

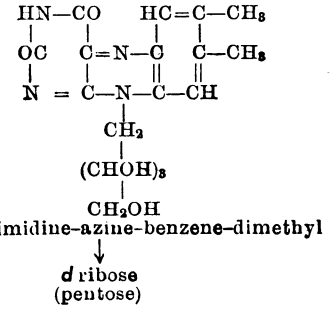
The colour reaction may be used for purified vitamins, but it is not yet applicable to vitamin in comparatively dilute form in foodstuffs. Several tests are used ; the most sensitive for solutions of the vitamin are the catatorulin test with pigeons' brain, essentially an application of the above, and Schopfer's micro-organism test.[27]

It must be noted that previous to this Reader and Orr-Ewing worked out a rather similar test with *S. corallinus*. Schopfer's test works to 0.01γ, and has been applied to blood by A. P. Meiklejohn. There still remains as standard the old curative test for pigeons showing the characteristic of opisthotonos, and the various tests utilizing growth, such as rat and pigeon maintenance tests, and the " heart abnormality " observation—namely, the bradycardial condition in rats used by L. J. Harris and his associates for estimating the vitamin.

The remainder of vitamin B, once vitamin B_2, has become complex, and has been divided already into two components—namely, lactoflavin and vitamin B_6. There are probably others. As previously mentioned, the relation of them to human nutrition is on less certain ground. My further remarks must be considered in this light.

THE FLAVINES

These must be distinguished from the flavones (allied to plant pigments) and compounds of quite different character, such as acriflavine. The structure is $C_{17}H_{20}N_4O_6$;



The flavines are thus allied to purines, pyrimidines, and nucleotides in a rather different way than is vitamin B_1. The compounds exhibit a beautiful yellow-green fluorescence, especially in ultra-violet light, which can be used as a means of estimation.

We know now that lactoflavin constitutes part of another essential oxidation system, the so-called yellow oxidation enzyme of Warburg ; the complete enzyme consists of lactoflavin phosphate united to a protein compound ; the whole resembles somewhat the porphyrin-iron globin, which constitutes haemoglobin. This fluorescent substance, together with others, is universally distributed. Evidently the lactoflavin part cannot be synthesized by the animal. It is very light-sensitive. In animals a regular effect upon growth follows withdrawal, and there are rather indefinite scurvy-like symptoms.[22] For rats, doses of 0.05 to 0.1 mg. are needed more than vitamin B_1. The compound is considered to be needed by dogs. We must keep our eyes open for its application to man, though Dann[23] claims that it has no effect on human pellagra.

VITAMIN B_6

This seems to be the real rat anti-dermatitis factor, and therefore must form the main constituent of the original vitamin B_2. (Its possible relation to pellagra in man must still be considered to be not properly defined.) In its absence we see in rats symptoms of very definite type—redness and swelling affecting first the paws and tips of ears and nose, a blood-stained mouth, and a spectacled loss of skin round the eyes. The factor is more stable to alkali and heat than other factors of the complex. There is some suggestion that it is also sensitive to light.

It is interesting to note that whereas liver contains both flavine and vitamin B_6, fish and cereals contain mainly B_6, while milk and white of eggs contain mainly flavine.[24] This suggests a real value for fish in our usual course dinner.

Other Possibilities

The foregoing is perforce sketchy, but should be enough to draw attention now to the main points in a field in which a very large number of people are engaged. There are several other problems upon the fringe of this one which cannot be neglected in this discussion.

The relation of acrodynia or pink disease to vitamin B_2 has been frequently raised from the time when Findlay[25] drew attention to the possibility ; some authors do not believe in it. The most remarkable resemblance in animals to clinical pink disease which I have seen was produced in some rats by Miss Reader in my department, in connexion with experiments with vitamin B_4. I still believe that there is some association between the other vitamins B and pink disease in children, but I am thoroughly alive to the difficulty of settling such a problem where clinical impression is the only guide. With crude vitamin B_1 concentrates Dr. Gibson and Mr. Stenhouse have observed in the Radcliffe Infirmary what they consider to be definite effects. I have also had favourable as well as unfavourable reports from other clinicians. More work on a wider scale is needed. The matter cannot be considered to be settled against the B complex until concentrated vitamins have been given.

It is also surely worth while to keep our eyes open for heart factors. C. W. Carter has devoted much time in my laboratory to the study of a heart block in pigeons due to vagal overaction, which is a dietary deficiency. Another interesting possibility rises from the suggestion that cataract in rats may be produced by diets deficient in vitamin B_2.[29]

The relation to human pellagra is very dangerous ground. Birch, Gyorgy, and Harris[24] have reported (1) that the condition of black tongue in dogs is not cured by vitamin B_6 or lactoflavin, and is therefore not due to these parts of the vitamin B_2 complex, and (2) that rats do not get dermatitis upon black tongue or human pellagra producing diets. From this they conclude that the black tongue and pellagra factors may be identical, but are certainly different from vitamin B_2. Other observers such as Zimmerman, Cowgill, Burnell, and Dann[26] have found that certain diets lacking in the vitamin B_2 complex produce marked demyelination of the peripheral nerves in dogs, the posterior and anterior spinal nerves, and the posterior columns of the spinal cord, without black tongue symptoms. There is clearly much work to be done to unravel this question ; it is a problem in which clinical and veterinary observation would be of value.

These remarks of mine are intended to add substance to the significance of nutrition in disease.

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A bronze bust of Dr. Emile Roux, presented to the town of Angoulême by the Académie de Médecine in the name of the National Roux Foundation, has recently been unveiled at Angoulême, when addresses were delivered by Professor Marchoux representing the Académie de Médecine, and Dr. Louis Martin, director of the Institut Pasteur of Paris.